The effect of individual genetic heterozygosity on general homeostasis, heterosis and resilience in Siberian larch (*Larix sibirica* Ledeb.) using dendrochronology and microsatellite loci genotyping

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ABSTRACT

The genetic mechanisms underlyingthe relationshipofindividual heterozygosity (IndHet)withheterosis and homeostasisare not fully understood. Such an understanding, however, would have enormous value as it could be used to identifytrees better adapted to environmentstress. Dendrochronologydata, in particular the individualaverageradial increment growth of wood measured as the averagetree ring width (AvTRW)and the varianceof tree ring width (VarTRW)were used as proxies for heterosis(growth rate measured as AvTRW)and homeostasis (stability of the radial growth of individual trees measured as VarTRW), respectively.These traits were then usedto test the hypothesisthatIndHetcan be used to predict heterosisandhomeostasis of individual trees.Wood core and needle samples were collected from 100 trees of Siberian larch (*Larix sibirica* Ledeb.) across two populationslocated in Eastern Siberia. DNA samples wereobtained from the needles of each individual tree and genotyped foreight highly polymorphic microsatellite loci. Then mean IndHetcalculated based on the genotypes of eight loci for each tree was correlatedwith the statistical characteristics of the measured radial growth (AvTRWand VarTRW)and the individual standardized chronologies. The analysis did not reveal significant relationships between the studied parameters. In order to account for the strong dependence of the radial growth on tree age the age curves were examined. An original approach was employed to sort trees into groups based on the distance between these age curves. No relationship was found between these groups and the groups formed based on heterozygosity. However, further work with more genetic markers and increased sample sizes is needed to test this novel approach for estimating heterosis and homeostasis.

Keywords: Dendrochronology; Tree ring width; Radial growth; Individual heterozygosity; Microsatellite markers; Heterosis; Homeostasis; Climate change; Environmental stress

Introduction

The concept of individual homeostasis in a heterogeneous environment as indicated by the low impact of environmental factors(temperature, precipitation, etc.) on individual development was first introduced by Walter Cannon (1929). It was further developed into the concept of developmental homeostasis (Dobzhansky and Wallace, 1953), genetic homeostasis (Lerner,

1954),developmental stability (Mather, 1953; Thoday, 1955) and phenotypicstability (Lewis, 1954).The concept was based onthe observationthat individualswith higherindividualheterozygosity(IndHet) were characterized bya more stable growth pattern andless impacted byenvironmental factors, such as, for instance,temperature and precipitation (see Livshits and Kobyliansky, 1985 for early review). The concept was revisited and reevaluated multiple times, but still needs additional studies and experimental data to improve our understanding of the molecular basis and genetic mechanisms underlying individual homeostasis and heterosis (see for more recent review Woolf and Markow, 2003; Hochholdinger and Hoecker, 2007; Fridman, 2015; Lippman and Zamir, D., 2007; Nicoglou, 2015; Peirson, 2015).

Stable growth pattern and the problem of individual responseto environmental stress should receive special attention in light of global climate change. Long-term changes in climates as well as short-term fluctuations in weather are of special concern for long-lived, sessile plant species such as forest trees, because unlike freely moving organisms, such as most animals and some plants they cannot purposefully search for a favorable habitat and move to it, and have to withstand environmental stresses during their lifetime as long as, for instance, 300-400 years on average and up tomaximum 750 years for Siberian larch (*Larix sibirica* Ledeb.) (Vaganov et al., 2006).Conifers, such as pine, larch and spruce, are the keystone species of the boreal forest ecosystems that could be both significantly affected by global climate change and at the same time play a very important role in the mitigation of climate change effectsdue totheir ability to store large amounts of carbon (Kasischke and Stocks, 2000; Soja et al., 2007; Nelson et al., 2008; Chen and Luo, 2015; Gauthier et al., 2015). Conifers have a substantial adaptive capacity at the individual tree level due to the high phenotypic plasticity and at the population level due to the high genetic variation (Hamrick, 2004; Santos-del-Blanco et al., 2013). However, genetic mechanisms of this high adaptability at both individual and population levels are still not fully understood. Siberian larch was selected for study here as it is one of the major boreal tree species in Eurasia (Kobak et al., 1996; Abaimov, 2010; Shuman et al., 2011).

We consider two main hypotheses for thegenetic mechanismsthat may explainwhy individualswith higherIndHetcould beless impacted byenvironmental factors and demonstrate higher heterosis: 1)overdominance (seereview by Hansson and Westerberg,2002), and 2) dominance, because highly heterozygous individuals by definition have lower levels ofinbreedingand less inbreeding depression(see,e.g., David,1999; Reed et al., 2012; Gonzalez-Varo et al., 2012; Abrahamsson et al.,2013). Both these genetic mechanisms could be responsible for the stablegrowth of individual trees with higher IndHet and their resistance to fluctuations in the environment, i.e. homeostasis can be associated with heterosis due to either the higher fitness of heterozygotes because of dominance (when the detrimental or less favorable recessive alleles that weaken the individual adaptability in homozygotes are masked and do not affect the individual fitnessin heterozygotes) and/or overdominance (when heterozygotes have higher fitness than any of homozygotes). Either case would lead to the natural selection of trees with higher IndHet, and one can expect that trees that are more resistant to (and more independent from) the environmental stress would have both a more stable development and a higher IndHet. Maladaptive seedlings and trees would occur in the population, however, as a genetic segregation load that could be a heavy price that a population would need to pay to maintain a high level of heterozygosity (Altukhov, 1991). Therefore, we expect also that there is an optimal level of IndHet. Exceeding this optimal level may lead to an increase of the segregation load and thus IndHet can be regulated by selection making extremely heterozygous trees less adaptive and less stable.

In addition, severalvariants ofcertain multimeric enzymes can be formed in heterozygotes, which acting together may be more efficientthan the single form of the enzyme foundin homozygotes (Berger, 1976).In this case,heterosisandhomeostasiscan bedue tooverdominance of heterozygotes. Moreheterozygous individualsare better adapted according to the theory of balancingselectionin favorof heterozygotes. The mechanisms ofheterosisandhomeostasisare poorlyunderstood, however, and available dataare very contradictory.

Both heterosisandhomeostasishave beenstudiedin different organisms, includingtree species and usingdifferent traits and geneticmarkers, such asallozymes(e.g., Ledig et al., 1983; Mitton and Grant, 1984; Strauss, 1986; Bush et al., 1987;Strauss and Libby, 1987; Zouros et al.,1988; Jelinski, 1993; Gonzalez-Varo et al., 2012), microsatellitesorso-calledsimple sequence repeats - SSRs (e.g., Abrahamsson et al., 2013; Zgaga et al.,2013),as wellas single nucleotide polymorphisms- SNPs (e.g., Govindaraju et al.,2009; Chelo and Teotonio, 2013).Correlation of IndHetwith various physiological, morphological and biochemicaltraits ofheterosisandhomeostasis(stable development)wasestimated in these studies.Traits used includedbilateral asymmetry(see Livshits and Kobyliansky, 1991; Parsons, 1992;Leung et al., 2000 for early reviews and more recent Kurbalija et al., 2011; Weisensee, 2013), growth rate (Ledig et al., 1983; Mitton and Grant, 1984; Strauss, 1986; Bush et al., 1987; Strauss and Libby, 1987; Zouros et al., 1988; Jelinski, 1993), and skeletal meristic traits (Zink et al., 1985).

The main objective of our study was to examine relationships between the level of heterosis and homeostasis measured using dendrochronology traits, such as the averagetree ring width (AvTRW)and the varianceof tree ring width (VarTRW),and IndHetmeasured with genome wide genetic markers, such as microsatellite loci (SSRs). In this initial study we usedrandom (and, therefore, likely intergenic) genomic SSRs that are supposedly selectivelyneutralgeneticmarkers. Microsatellite loci were chosen because they are highly informative and relativelyinexpensive for measuring genome-wideindividual heterozygosity (but see Väli et al., 2008). They have high mutation rate, high levels of polymorphism, relativelyuniform distributionacross the genome, broad representation, and are relativelysimpleto detect andto genotype (e.g., Schlötterer, 2000).

In our study we used a novel approach to address homeostasisfrom perspectives oftwo disciplines-dendrochronologyand populationgenomics(Gonzalez-Martinez et al.,2006; Krutovsky and Neale, 2005; Krutovsky,2006).Thisapproach allows us tomore effectivelystudy theadaptabilityof natural populationsto globalclimate change(King et al.,2013), and how genetic variationmay be affected(Pauls et al.,2013). For the first time here we propose to usetree ring data

to estimate stability and homeostasis.TheAvTRW and VarTRW parameters are particularlyusefulbecausetheylikely correlatewith veryimportantenvironmentaland climaticfactors such asprecipitation, temperature, and length of growthperiod (Vaganov et al., 1996, 1999, 2006).

The maintask inour studywas to test thehypothesisthat IndHetis associatedwith AvTRWandVarTRW.In the earlygenetic studies some evidencewas obtained suggestingthatIndHet is positively associated withheterosis –a higher viability and strongeradaptive traitswere observed inhybridsobtained from crossingparents that weregeneticallydifferentanddistantfrom each other. It was expressed as higher resistance toenvironment change or stress, increased growth rate and biomass growth, etc. (Schnable and Swanson-Wagner, 2009; Schnable and Springer, 2013; Feng et al., 2015).

Ifmoreheterozygoustreesare characterized by a more stablehomeostasis, then theirdevelopment should be less dependent onthe environment. Therefore our expectation was to find a negative correlationbetweenIndHetand VarTRW.If AvTRW can be considered as anadaptive trait, then one can expect a positive correlationbetween IndHetand AvTRW due to heterosis.

There may, however, be an optimal level of IndHet resulting in nonlinear relationships between IndHet with AvTRW and VarTRW. HighIndHetcan leadto an increased the segregationload in thepopulationand causean imbalancein the individual development.On the other hand, lowIndHetmay resultfrom inbreeding,in whichfrequency of homozygotesforunfavorablerecessive alleles increase.This in turncould adversely affect AvTRW, causing a negative correlation ofthe level ofhomozygositywith AvTRWandalso disrupthomeostasis. The latter would be manifested as a positivecorrelation between the levelof homozygosityandVarTRW.Dendrochronological and genetic data werecollected for the same individual trees to assessAvTRW,VarTRW, andIndHet and to test these hypotheses.

Materials and methods

Plant material

Wood cores of Siberian larchwere collectedin July, 2014,from the following two populationsin theShira region ofKhakasia:1)the predominantly larch forestmixed with pine and some birch trees on a gentle southeastern slope (2-5°, 600-700 m a.s.l.) near the Shira-Berenzhak highway (this population is denoted as "BER"; Fig. 1);and 2) the larchlight forest on a steep western slope (up to 30°, 600-800 m a.s.l.)from the top to the base of the hill in the vicinity of theEfremkino village (this population is denoted as "EFR"; Fig. 1). The distance between the BER and EFR populationsis approximately25km.Fifty trees of approximately similar age were randomly sampled in each population according to the dendrochronological principles (standing apart mature trees with minimal nonclimatic impacts) (Cook and Kairiukstis, 1990), taking also into account availability of live branches to collect needles for DNA isolation.Two wood cores were taken from each tree to measure tree rings.Needles were also collected fromthesametreesfor DNA isolation and genotyping.

Tree-ring width data processing

Initialextraction ofwood coresandmeasurement of the tree-ringwidth (TRW) were performed using standard procedures(Cook and Kairiukstis, 1990).Asemi-automateddevice LINTAB-5and a specializedprogramTSAP Win were employed (Rinn,2011). Cross-dating of the original serieswas performed using the COFECHA program(Holmes,1998). About fivecores from each populationwerepartially broken because the larchwoodin the study area was particularly brittle. Consequently,thetime seriesobtainedfrom these cores were missing from two to three rings. For further work the estimatesfor these cores wereadjusted using the ARSTAN program (Cook, Krusic, 2005). This was accomplished by constructing a 20-year spline, on which the TRW fluctuationsobserved on the duplicatecorefrom the sametree were superimposed. The mean time series for each tree were obtained by averagingmeasured TRW values for duplicate cores (Fig.2).

Most cores did not pass through the pith due to the frequently observed offset of the pith from the geometric center of the tree cross-section and the sampling imperfection. The pith wasalso damagedin 2-3 trees per population.We estimated the numberof missinginnermost rings (pith offset, PO)usingthe radius of curvatureand the widthof theinnermostavailable rings, while taking into

account thecross-dating results for duplicate cores from the same tree (Duncan, 1989, Esper et al., 2009). Usingthe ARSTAN program we plotted the age trend curves for each tree using the following two approaches: 1)spline having the length equal to67% of the length of the series and2)an exponential function or in the case of this resulting in negative valuesonthe exponential curve, a linear function.

The calculation of the distance between the agecurves$A(\mathrm{t})$was carried outfor the age interval6-127years (using the median *Me* of the parameter*PO*and the cambialage*T*of the treesmeasured in the year 2014).The distances Δ_{ij} were calculatedforeach pair of*i* and*j*treeusing the formula:

$$\Delta_{\mathrm{ij}} = \frac{1}{\mathrm{t}_2 - \mathrm{t}_1 + 1} \sum_{\mathrm{t}=\mathrm{t}_1}^{\mathrm{t}_2} \left| \mathrm{A}_{\mathrm{i}}(\mathrm{t}) - \mathrm{A}_{\mathrm{j}}(\mathrm{t}) \right|, \quad (1)$$

where $t_1 = \max(PO_i, PO_j, Me(PO))$ and $t_2 = \min(T_i, T_j, Me(T))$are the commonborders for the considered trees in the certain age interval,taking into account the aboverestrictions.The resulting tableof the distanceswas employed to perform hierarchical clusteranalysis of the localset oftrees. The clusteringat each step was performedusing the method ofcomplete linkage.

Standardization of the raw tree-ring width data was processed in two steps with ARSTAN. At the first step, age trends described above were removed, thus standard (*std*) individual series and generalized (averaged) chronologies were obtained. At the second step, we removed autocorrelation of the first order (*ac1*) and obtained residual (*res*) individual series and chronologies.

Statistical characteristics of individual series and chronologies used included mean value (*mean*, that is AvTRW for the raw data), standard deviation (*stdev*, that is VarTRW for the raw data),mean coefficient of sensitivity (*sens*),autocorrelation of the first order (*ac1*),expressed population signal (*eps*), interseries average correlation coefficient (*rbar*), and correlation of individual series with their master chronology (*R*). Significance of differences between different groups of trees was tested using Student's *t*-distribution.

Climatic data

Monthlyclimatic datafordendroclimatological analysis were obtained from the Climatic Research Unit (CRU) database (http://climexp.knmi.nl/selectfield_obs2.cgi)fora gridwith a step of 0.5°forthe four points that are closest to thedendrochronologicalpolygons(Fig.1) for the period1901-2014. The following data wereused: theaverage temperature, total precipitation, and the Palmer Drought Severity Index (PDSI).Climate variableswere compared at different points, as well as with the instrumental data from the weather station"Shira" for temperature(1966-2012)and precipitation(1937-2012).Correlation coefficientswere calculatedfor the followingperiods:September-November, December-February, March-May, June-August andfor the full-year period from Septemberto August(Table1).

The interannual changesof temperature and precipitation for the most importantsummer period are illustrated in Fig. 3.While the CRU dataare well-correlated among each other, the correlation with the data fromthe weather station “Shira”is much lower, especially for precipitation.Thisdiscrepancy maybe because 1) the CRUdata were obtained by interpolationfrom other sources, possibly reflecting regional climate rather than weather at a specific point or2) the possibility of inaccurate instrumentation or human error at the weather station during a certain period. We decided to use the CRU climatic datafor further analysis because these data havelonger duration and are expected to have higher reliability over the full period.

The BER sampling populationis located 7km from grid point3, whereas the sampling populationEFR is3 kmfrom the center of the area(point8)bounded by the neighboringgridpoints. For the climaticresponse analysis we useddata for grid point3 for the BERchronologies and the averaged data forpoints1-4 for the EFR chronologies.

Genotyping with nuclearmicrosatellite loci

To estimategenetic polymorphismof the two populationsof Siberian larchand individual tree heterozygosity,we usedthe eight best performing and the most polymorphic nuclearmicrosatellite loci (SSRs)that werepreviously developedfor Japanese larch(*L. kaempferi* Sarg.) -loci*bcLK*, and for alpinelarch(*L. lyallii* Parl.)and westernlarch(*L. occidentalis* Nutt.) -loci*UAKly* and *UBCLX* (Table2),

and then adaptedfor theSiberian larch(Oreshkova et al.,2013). Thecharacteristics of these markers and the PCR conditions of their amplificationare presented inTable 2.

Individual samplesof total DNAwereextracted from100-200mgof needles per tree. Extractionswere performedaccording to the standardprotocol forplant tissuesusingcetyltrimethylammonium bromide, CTAB(Devey et al., 1996).

The fragment analysis and sizing of the amplified individual allelesof the microsatellitelociand theirgenotypingwere doneusing6% polyacrylamide gel electrophoresis(PAGE) inTris-EDTA-borate electrodebuffer.Gelswere stainedinethidium bromide solution and visualized using the system of gel documentation. The fragmentlengthswere determinedby comparisonwith the standardDNA ladder (plasmidpBR322 DNAdigested by the HpaII restriction enzyme) using the Photo-Capt software.To more precisely determine thelengths of the PCR fragments (microsatellite alleles) multiplecomparisonsof variantsof eachlocuswere performed by running them onthe samegel.Genetic diversityparameters including individual heterozygosity were estimatedusing the GenAlEx 6.41 software (Peakall and Smouse,2006).

Correlation Analysis

All relationships between variables were analyzed using Pearson's correlation coefficients.Significance of correlation was tested using Student's *t*-distribution. We also applied multifactorial analysis of variance using the Variance Components ANOVA/ANCOVA module in the STATISTICA software (StatSoft Inc., Tulsa, OK, USA) to estimate relationship of IndHet with AvTRW and VarTRW (using IndHet as a dependent variable, population as fixed effect, and AvTRW and VarTRW as random effects), but it gave results similar to the correlation analysis, therefore, these data are not presented here.

Results

Genetic variation was high in both populations across all loci, varying from 3 to 15 alleles per locus (Table 3). Observed heterozygosity (H_o) varied from 0.040 to 0.560 per locus and was 0.315 and 0.260 on average for all loci in BER and EFR populations, respectively.

Both parameters AvTRW and VarTRW hadpositive, but weak and statistically nonsignificant correlations withIndHet (Table 4, Fig.4).At thesame time,AvTRW and VarTRWwere positivelycorrelatedat a highly significant level. Relationships of IndHet were estimated using absolute values formeasured parameters (*raw*) of the individual seriesof radialgrowth, as well as with two types of standardized (*std* and *res*) parameters (Table 5, Fig.5). All correlation coefficients were close to zero and nonsignificant.

Since the radial growthlargelydepends on thetree age,a phenomenon referred to as theage trend, we also compared thegroups of trees characterized by differentlevels of IndHet with the groups (clusters) of trees characterized by different agecurves,determined by hierarchical classification using two methods of age curves estimation (spline / exponential function).Theobtained age curvesand the depth ofthe dataset aligned by the cambialage,i.e.,the number of treesfor each age, are shown in Fig. 6, the cluster subsets are shownin Figures7 and 8, and the dendrogramsof classification are shown in Fig.9. Differentmethods of calculating the agecurvesyieldedsignificantlydifferent results of classification, although certain common patternsmay be found. Nevertheless, no commonpatterns in thedistribution of trees with differentIndHetwere found in either case.

Each population(BER and EFR)was then partitioned intotwo subsets after removingtreesyounger than 50 years from the analysis. The first subset “low IndHet” – with the indexof individualheterozygosityin the range of 0-0.25, and the second subset “high IndHet” –with IndHet in the range of 0.375-0.75.For eachsubsetstandard dendrochronological procedures were then performed,andthe generalizedstandard(*std*)and residual(*res*)chronologies were obtained.The statistical characteristicsof the chronologies obtained using theARSTAN software areshown in Table6. Foreach subset, standarddendroclimatologicalanalysis was carried out.Correlation

coefficientsof thechronologieswiththe monthly total precipitation,average temperatureand the PDSI were found to be significant for some months (Fig.10).

Discussion

The highly significant and positivecorrelation between AvTRW and VarTRW was interesting. This phenomenon can be explained if under unfavorable conditions most (if not all) trees grow slower regardless of their genotype, but under favorable conditions some trees may respond better via increased radial growth.

It is difficult todraw a conclusionabout the relationships of AvTRW and VarTRW parameters withIndHet based on the data presented here.Although the correlations were nonsignificant, they were nonlinear rather than linear (Fig. 4). Therefore, the effect of individual heterozygosity could be very complex, and there may be an optimal intermediate level, when low individual heterozygosity could be as detrimental as a very high value (Altukhov et al., 1986; Altukhov, 1996, 1998, 1999; Altukhov and Sheremet'eva, 2000; Altukhov and Moskaleichik, 2006; Olano-Marin et al., 2011; Thoß et al., 2011).

Attempts to reveal the relationshipsbetween IndHet and individual series statistical characteristics and age curve groups did not give significant results. Use of generalized chronologies of subsets with low and high IndHet was more successful. The most significant and stable differences were found for expressed population signal (*eps*), which was higher for more heterozygous chronologies at all stages of standardization (Table 6). The same but less significant regularity was observed for the interseries correlation coefficients (*R*) and sensitivity (*sens*) coefficients. These patternssuggesta trend towardsmore pronouncedcommon external signalsin treeswith higherheterozygosity because both *R* and *eps*are measures of common variation of individualgrowth series in the chronology, especially since *eps* can be interpreted as a measure of closeness between individual series and theoretical chronology of entire population (Wigley et al., 1984).As common environmental factors become more extreme, the populationsexhibit a higher

synchrony in growth patterns of individual trees and thus the common signal (Cook, 1985; Briffa and Jones, 1990).In the same environment, a common signal also depends on tolerance of plants to local conditions (Merian and Lebourgeois, 2011).Autocorrelation (*ac1*) intheheterozygouschronologies, on the contrary, was lower (althoughthis differencewas significantinonly one population): that is, the radial growthinthe current yearwas less dependent ongrowth inthe previous year. Therefore,on the basis ofidentifiedtrends, we can assume thatfor trees withhigherheterozygosity there was a more pronouncedeffect of factors common for the entire population(climate, general characteristics ofthe landscape and thesoil), especially climatic variables with their high-frequency variation. For less heterozygous trees, the impact ofindividualstress factors, such as microenvironment andcompetitive relationship, was more important, which can be cautiously interpreted as their individual development is less stable.

Climatic response varied depending on heterozygosity. There wasa stronger negativeresponse to thewarm season temperatures for the data subsetswith highIndHetin both populations anda stronger positiveresponse to thePDSI and the spring-summer precipitation, as a factor decreasing water deficit stress in plants, inthe BER population. On the contrary, in the more humid and thus less extreme environmental conditions ofthe EFR population, the positive effect of increased precipitation and less severe drought (PDSI) was more pronouncedfor the data subset with low IndHet. The dendroclimatic analysis,however, generally confirmed an expected pattern of positive relationship between heterozygosity and common signal strength in moderately extreme conditions of water availability.

The lack of correlation between IndHet and characteristics of radial growth can be explained by the ascertainment bias caused by typically selecting only the most polymorphic microsatellite markers in the genome, which may lead to reduced sensitivity for judging genome-wide levels of genetic diversity. Väli et al. (2008) tested this potential limitation of microsatellite-based approaches by correlating nucleotide diversity in noncoding regions of eight different carnivore populations assessed by sequencing 10 introns (5.4–5.7 Kb) in 20 individuals of each population

with mean multilocus heterozygosities based on microsatellite genotyping (10–27 markers) of the same animals. Although there was a positive correlation between microsatellite marker heterozygosity and nucleotide diversity at the population level, no significant correlation was found at the individual level. These results imply that the variability of microsatellite marker sets typically used in population studies may not accurately reflect the underlying genomic diversity. This suggests that researchers should consider using resequencing-based approaches for assessing genetic diversity when accurate inference is critical, as it maybe in our case.

Another problem could be associated with a relatively high frequency of null-alleles that can mask heterozygotes. The high *F*-values observed in several loci in both populations (Table 3) can be a signature of null-allele presence. Inbreeding can also inflate *F*-values, and self-pollination seems higher in larch compared to other conifers (Knowles et al., 1987; Oreshkova et al.,2013), but it cannot explain uneven distribution of *F*-values across loci.

SSR markers alone did not allow us to discriminate two main hypotheses: overdominance vs. dominance, but only to test the association of IndHet with the average tree-ring width (AvTRW) and with the variance of the tree-ring width (VarTRW) used as proxy traits for heterosis and homeostasis, respectively. In the following studies we plan to use also supposedly adaptive genetic markers, i.e. microsatellites closelylinked with functionalandadaptivegenes, and sequence data – that are SNPsin the coding (preferably nonsynonymous SNPs) regions, as well as supposedly selectively neutral SNPs in noncoding regions for comparison.A description of thedifferent types ofgenomicmarkersproposedin our study and alsorecommendedfor the study ofthe impact of globalclimate changeon the genetic variabilityof populations and species is provided in Angeloni et al. (2012).

Conclusions

Dependence of some radial growth characteristics of Siberian larch trees on their individual heterozygosity was investigated. Application of different approaches demonstrated that partitioning

the populations into two groups (subsets) with low and high individual heterozygosity, respectively, and the subsequent comparison of their chronologies provided additional valuable information. It can be assumed that radial growth of trees with high IndHet responded more strongly to the climatic changes because of their faster recovery after extreme stress. On the contrary, radial growth of trees with low IndHet is more autoregressive and is more affected by continuously acting stress factors. In our further work we plan to increase the number of loci to make them more genome wide for more accurate estimation of individual heterozygosity and for better detection of environmental signals.

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Table 1

Correlations between various climatic data series for the time interval 1966-2012.

Sites	Temperature					Precipitation					PDSI				
	Fall	Winter	Spring	Summer	Year	Fall	Winter	Spring	Summer	Year	Fall	Winter	Spring	Summer	Year
1-2	0.996	0.998	0.998	0.991	0.998	0.967	0.968	0.950	0.928	0.954	0.853	0.823	0.891	0.924	0.908
1-3	0.993	0.996	0.998	0.990	0.996	0.886	0.843	0.843	0.834	0.896	0.781	0.688	0.783	0.880	0.815
1-4	0.999	0.999	0.999	0.998	0.999	0.961	0.930	0.948	0.945	0.965	0.888	0.824	0.923	0.937	0.915
2-3	0.999	0.999	0.999	0.998	0.999	0.954	0.932	0.949	0.954	0.966	0.818	0.776	0.877	0.921	0.896
2-4	0.997	0.999	0.997	0.992	0.998	0.963	0.958	0.924	0.938	0.949	0.815	0.758	0.916	0.917	0.898
3-4	0.996	0.998	0.998	0.993	0.998	0.947	0.968	0.893	0.942	0.953	0.918	0.865	0.914	0.938	0.920
1-Mean	0.998	0.999	0.999	0.997	0.999	0.973	0.960	0.965	0.951	0.972	0.936	0.908	0.943	0.964	0.948
2-Mean	0.999	1.000	0.999	0.998	0.999	0.992	0.990	0.988	0.984	0.987	0.925	0.913	0.963	0.970	0.965
3-Mean	0.998	0.999	0.999	0.998	0.999	0.967	0.960	0.952	0.963	0.975	0.936	0.908	0.934	0.965	0.947
4-Mean	0.999	1.000	0.999	0.998	1.000	0.987	0.989	0.969	0.984	0.986	0.964	0.941	0.982	0.978	0.974
1-Shira	0.965	0.974	0.964	0.901	0.977	0.328	0.287	0.276	0.372	0.362					
2-Shira	0.967	0.970	0.965	0.914	0.975	0.359	0.403	0.339	0.514	0.420					
3-Shira	0.967	0.971	0.968	0.920	0.977	0.372	0.610	0.441	0.611	0.479					
4-Shira	0.967	0.975	0.967	0.911	0.979	0.417	0.507	0.433	0.516	0.452					
Mean-Shira	0.968	0.973	0.967	0.914	0.977	0.376	0.460	0.381	0.523	0.439					

Table 2

Microsatellite loci genotyped in Siberian larch in this study.

Locus	Motif	Annealing T (ºC)	Number of alleles[a]	Fragment size, bp	Reference
bcLK056	$(AG)_{20}$	Touchdown 63-53ºC	12/10	140-200	IsodaandWatanabe, 2006
bcLK066	$(TG)_{12}$		5/4	140-172	
bcLK224	$(AG)_{17}$		9/4	130-168	
bcLK260	$(TG)_{14}(AG)_9$		5/5	80-126	
bcLK232	$(AG)_{19}$		10/4	135-178	
bcLK235	$(TC)_9(AC)_2AG(AC)_{14}$	58ºC	9/15	168-220	
UBCLXtet-1-22	$(TATC)_9(TA)_{12}$		8/3	175-250	Chenetal.,2009
UAKLly6	$(GT)_{17}$		13/9	212-264	Khasaetal., 2000, 2006

[a]First number is a number of microsatellite alleles published earlier; second one is a number of alleles discovered in this study.

Table 3

Genetic variation of eight microsatellite loci in two Siberian larch populations.

Population[a]	Parameter	*bcLK056*	*bcLK224*	*bcLK066*	*bcLK260*	*bcLK235*	*UBC-1-22*	*UAKLly6*	*bcLK232*	Mean±SE
	N_a	10	4	4	5	15	3	9	4	6.8±1.5
	N_e	6.2	2.8	1.4	2.1	8.8	1.2	5.6	1.7	3.7±1.0
BER	H_o	0.340	0.180	0.260	0.340	0.560	0.040	0.380	0.420	0.315±0.056
	H_e	0.839	0.637	0.270	0.517	0.886	0.185	0.821	0.407	0.570±0.095
	F	0.595	0.717	0.037	0.343	0.368	0.784	0.537	-0.032	0.419±0.106
	N_a	9	3	4	5	9	3	7	3	5.4±0.9
	N_e	5.4	1.8	1.2	1.4	4.3	1.4	4.3	1.2	2.6±0.6
EFR	H_o	0.420	0.200	0.180	0.120	0.440	0.260	0.320	0.140	0.260±0.043
	H_e	0.816	0.455	0.168	0.287	0.768	0.295	0.767	0.165	0.465±0.099
	F	0.486	0.561	-0.073	0.582	0.427	0.120	0.583	0.154	0.355±0.089
Mean ±	N_a	9.5±0.5	3.5±0.5	4.0±0.0	5.0±0.0	12.0±3.0	3.0±0.0	8.0±1.0	3.5±0.5	6.1±0.9
standard	N_e	5.8±0.4	2.3±0.5	1.3±0.1	1.7±0.3	6.5±2.2	1.3±0.1	4.9±0.7	1.4±0.2	3.2±0.6
error (SE)	H_o	0.380±0.040	0.190±0.010	0.220±0.040	0.230±0.110	0.500±0.060	0.150±0.110	0.350±0.030	0.280±0.140	0.288±0.035
over both populations	H_e	0.828±0.011	0.546±0.091	0.219±0.051	0.402±0.115	0.827±0.059	0.240±0.055	0.794±0.027	0.286±0.121	0.518±0.068
BER & EFR	F	0.540±0.055	0.639±0.078	-0.018±0.055	0.463±0.120	0.398±0.030	0.452±0.332	0.560±0.023	0.061±0.093	0.387±0.067

[a] 50 trees were genotyped in each population. N_a – number of different alleles; N_e – number of effective alleles = $\frac{1}{\sum_{i=1}^{n} p_i^2}$; H_o – observed heterozygosity = $\frac{\text{number of heterozygotes}}{N}$; H_e –expected heterozygosity = $1 - \sum_{i=1}^{n} p_i^2$; F – fixation index = $(H_e - H_o)/H_e = 1-(H_o/H_e)$; where N is number of trees genotyped, and p_i is the frequency of the ith allele in the population.

Table 4

Correlations between average tree ring width (AvTRW), variance of tree ring width (VarTRW) and individual heterozygosity of trees (IndHet).

Population[a]	**Parameter**	**AvTRW/VarTRW**	**IndHet/AvTRW**	**IndHet/VarTRW**
BER	*R*	0.805	0.215	0.265
	P	0.000*	0.134	0.063
EFR	*R*	0.660	0.203	0.203
	P	0.000*	0.156	0.158
Combined (BER+EFR)	*R*	0.726	0.146	0.122
	P	0.000*	0.147	0.225

[a] 50 trees were genotyped in each population. *R* - correlation coefficient, *P* - significance level (**P*< 0.001).

Table 5

Correlations of individual heterozygosity (IndHet) of trees with their radial increment growth statistics in two populations (BER and EFR).

Population	**Parameter**	**raw**					***std***			***res***	
		mean (AvTRW)	*stdev*(VarTRW)	*sens*	*ac1*	*R*	*stdev*	*sens*	*ac1*	*stdev*	*sens*
BER	*r*	0.215	0.222	0.109	-0.142	-0.173	0.045	0.088	-0.117	0.047	0.050
	p	0.134	0.122	0.449	0.325	0.231	0.757	0.542	0.420	0.744	0.732
EFR	*r*	0.172	0.202	0.119	-0.035	0.190	0.017	0.115	-0.068	0.059	0.006
	p	0.234	0.159	0.412	0.809	0.186	0.907	0.426	0.637	0.684	0.969
Combined	*r*	0.126	0.111	0.054	-0.062	0.024	0.023	0.048	-0.038	0.005	0.002
(BER+EFR)	*p*	0.213	0.272	0.597	0.540	0.814	0.822	0.635	0.710	0.964	0.985

r - correlation coefficient with IndHet, *p*- significance level (other parameters and abbrviations are explained in Materials and methods).

Table 6

The mean values and standard deviations (mean ± standard deviation) of statistics for original (*raw*) and standardized (*std* and *res*) radial growth chronologies of two populations (BER and EFR) partitioned for groups with low and high individual heterozygosity (IndHet) of trees.

Type of chronology	**Statistics**	**Chronology**			
		BER		**EFR**	
		low IndHet	**high IndHet**	**low IndHet**	**high IndHet**
raw	mean(AvTRW)	1.42±0.63	1.37±0.68	0.80±0.72***	1.85±0.77**
	stdev(VarTRW)	0.74±0.26	0.75±0.34	1.13±0.45	1.11±0.45
	sens	0.36±0.07**	0.39±0.07**	0.44±0.11	0.47±0.10
	ac1	0.66±0.14	0.65±0.15	0.67±0.14**	0.59±0.12**
	rbar	0.57±0.12*	0.62±0.15*	0.56±0.10	0.59±0.08
	eps	0.94±0.05***	0.96±0.02***	0.94±0.04***	0.97±0.01***
	R	0.74±0.10	0.75±0.10	0.69±0.14***	0.76±0.08***
std	*stdev*	0.48±0.09*	0.51±0.12*	0.53±0.11	0.51±0.10
	sens	0.36±0.07**	0.39±0.07**	0.43±0.11	0.46±0.10
	ac1	0.57±0.14	0.56±0.17	0.54±0.11***	0.43±0.14***
	rbar	0.56±0.13**	0.62±0.15**	0.57±0.09**	0.62±0.09**
	eps	0.93±0.05***	0.96±0.02***	0.94±0.03***	0.97±0.01***
res	*stdev*	0.37±0.06*	0.39±0.07*	0.43±0.09	0.45±0.09
	sens	0.43±0.08*	0.46±0.08*	0.48±0.12	0.49±0.10
	rbar	0.62±0.09	0.65±0.10	0.60±0.07	0.62±0.09
	eps	0.94±0.06*	0.96±0.03*	0.95±0.03**	0.97±0.01**
Number of cores		41	54	55	27

Significance level of differences between groups with low and high individual heterozygosity: *$p < 0.10$, **$p < 0.05$,***$p < 0.01$.

FIGURELEGENDS

Fig. 1. Mapof the study area. Numbers1-4 and 8 indicategrid points for climatic dataCRU, 5 - the middle ofthe squaregridfor meteorological station"Shira", 6 and 7 - dendrochronologicalpolygonsfor populations Efremkino (EFR)and Berenzhak (BER), respectively.

Fig. 2. Tree ring width(TRW) of the individual treesand the local measuredchronology(red line)in the BERpopulation along the years measured.

Fig. 3. Summertemperatureand precipitationin the study area based on data fromdifferent sources (see Material and methods).

Fig. 4. Correlation ofthe averagetree ring width (AvTRW)and the varianceof tree ring width (VarTRW) with individual heterozygosity(IndHet) of trees, andAvTRW vs. VarTRW measured in two populations (50 trees each) combined.

Fig. 5. Scattering diagramsof the studied statistical characteristicsfor the measuredand standardizedindividualchronologiesof radial increment growthwith the individual heterozygosity (IndHet) of treesparameter.

Fig. 6. Agecurves for the population BER,calculatedusing different methods. A(t), mm –age curve (function of age trend) of radial growth in millimeters, N –number of trees for each age.

Fig. 7. Clusters of agecurves calculatedas splines.

Fig. 8. Clusters of agecurves calculatedasexponential andlinear functions.

Fig. 9. Hierarchicaldendrogramsfor the BERpopulation dataset(clusterization is based onthe agecurves).

Fig. 10. The climaticresponsein the chronologies ofthe two localpopulation datasets(BER and EFR) withlower and higherheterozygosity.Dotted line indicates thesignificance threshold for $P < 0.05$.